An integrated fingerprinting and kinetic approach to accelerated shelf-life testing of chemical changes in thermally treated carrot puree

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Abstract

To have a better understanding on chemical reactions during shelf-life, an integrated analytical and engineering toolbox: "fingerprinting-kinetics" was used. As a case study, a thermally sterilized carrot puree was selected. Sterilized purees were stored at four storage temperatures as a function of time. Fingerprinting enabled selection of volatiles clearly changing during shelf-life. Only these volatiles were identified and further zoomed into. Next, kinetic modelling was performed to investigate the suitability of these volatiles as quality indices (markers) for accelerated shelf-life testing (ASLT). Fingerprinting enabled selection of terpenoids, phenylpropanoids, fatty acid derivatives, Strecker aldehydes and sulfur-compounds as volatiles clearly changing during shelf-life. The amount of Strecker aldehydes increased during storage, whereas the rest of the volatiles decreased. Out of the volatiles, based on the applied kinetic modelling, myristicin, α-terpinolene, L-β-pinene, α-terpineol and octanal were identified as potential markers for ASLT.

Keywords

Thermal sterilization; carrot puree; accelerated shelf-life testing; headspace GC-MS fingerprinting; kinetic modelling; chemical reactions.

Abbreviated running title

Fingerprinting-kinetics of shelf-life testing of sterilized carrot

1. INTRODUCTION

Theoretically, when based on microbiological safety, sterilized food can be stored indefinitely. However, the best before date is limited due to chemical changes whether or not triggered during processing and incessantly taking place during shelf-life (van Boekel et al., 2010). Considering consumer expectations, quality should be maintained at a targeted level during the period between processing and purchase as well as between purchase and consumption. There is a need for studies which not only take into account quality changes during processing, but also as a function of shelf-life. For practical reasons, especially when the actual storage time is long, shelf-life studies are based on accelerated shelf-life testing (ASLT) techniques that considerably shorten the process of obtaining the necessary experimental data (Mizrahi, 2000). ASLT works with a basic assumption that the effects of extrinsic parameters (mostly temperature) on the rate of deteriorative reactions can be quantified by applying the principle of chemical kinetics (Labuza & Taoukis, 1990; Mizrahi, 2000; Robertson, 2000). In other words, with the use of increased temperature as abuse condition and assuming that reactions follow Arrhenius kinetics, deterioration rates at ambient/normal distribution conditions can be extrapolated from those of elevated temperatures (Hough, Garitta, & Gomez, 2006; Corradini & Peleg, 2007).

Traditionally, quality investigations during shelf-life have been performed using an univariate approach in which the change in food quality was tailored either with the loss of predetermined quantifiable quality indices such as nutrient or by the formation of an undesirable off-flavor and/or discoloration of compounds (Sithole, McDaniel, & Goddik, 2005). This strategy is the most straightforward way to address such quality investigations, and the corresponding results are undoubtedly of great value. However, fixating on known quality aspects entails that possible different effects are overlooked. Since food degradations are caused by the interaction of many attributes, more comprehensive results can be collected, that former methodologies were not

capable of doing, for example using currently existing food chemometric tools (Saavedra, Cordova, Galvez, Quezada, & Navarro, 2013).

In the present work, to have a better understanding on chemical reactions during shelf-life, the use of an advanced analytical method, relevant data preprocessing methods, multivariate statistical techniques and kinetic models were integrated to develop an analytical and engineering toolbox, called "fingerprinting-kinetics". As a case study, a thermally sterilized carrot puree was selected. Sterilized purees were stored at four different temperatures: 20 °C, 28 °C, 35 °C and 42 °C. In this work, quality changes linked to the volatile fraction were studied. Volatiles are often linked to process-induced reactions and have a major contribution to food flavor. Volatiles, being regularly degradation products of major food components (e.g., sugar, fat, nutrients), they can be approached as witnesses for what is happening in a complex food system. The volatile fraction of the samples was analyzed with a headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting procedure as a function of storage times and temperatures (kinetics). This approach considers all compounds detected in the investigated food fraction. Within a fingerprint procedure at the start of the analysis all the compounds are unknowns, it has been called an "untargeted approach" before (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014). The amount of data generated using a HS-SPME-GC-MS analysis might be overwhelming. Multivariate statistical data analysis (MVDA) techniques are most appropriate for extracting important information out of these large data sets by reducing the dimensions of the multivariate data to a few manageable dimensions.

The objective of the present work was twofold. As a first objective, the potential of fingerprinting was used as fast screening technique for monitoring chemical changes during shelf-life. In other words, fingerprinting could enable selection of volatiles of which detected amounts are changing the most during shelf-life. Only these molecular entities were identified

and further zoomed into (to link them to possible reaction pathways). The power of fingerprinting to identify important reaction pathways within a complex of chemical changes was clearly demonstrated in our previous studies (Vervoort et al., 2012; Kebede et al., 2013; Kebede et al., 2014a; Kebede et al., 2014b).

Since conducting shelf-life study at the normal storage temperature can be quite resource consuming, as a second objective of this work, the potential of ASLT was investigated. Based on the data from the fingerprinting as a function of storage times and temperatures, a kinetic modelling was performed to investigate the reaction kinetics of volatiles (selected by the fingerprinting approach) at different storage temperatures. By evaluating the estimated kinetic parameters, the suitability of these volatiles as quality indices (markers) for ASLT was investigated (**Fig. 1**).

2. MATERIALS AND METHODS

2.1. *Sample preparation*

A single batch of freshly harvested carrots (cv. *Nerac*) was purchased at a local market. The carrots were carefully washed, peeled and cut into standardized cylindrical pieces of approximately 1 cm thickness. The carrot cubes were packed into low-density polyethylene bags. To prevent enzymatic reactions during processing, storage and thawing, the packaged carrots were blanched at 95 °C for 8 min in a water bath (Haake W15 DC-10, Clausthal-Zellerfeld, Germany). The blanching conditions were validated using a qualitative and quantitative peroxidase test (Adebooye, Vijayalakshmi, & Singh, 2008). After blanching, the plastic bags were immediately cooled in ice water for 10 min, frozen in liquid nitrogen and stored in a freezer at -40 °C until processing. Prior to processing, the samples were thawed overnight at 4 °C. In order to prepare the puree, deionized water was added to the blanched carrot, blended for 1 min using a Buchi mixer (B-400, BUCHI, Switzerland) and further homogenized by high pressure

homogenization (at 1000 bar while temperature maintained < 4 °C) (Panda 2K, Gea Niro Soavi, Mechelen, Belgium). The sample preparation steps are schematically listed in **Fig. 1**.

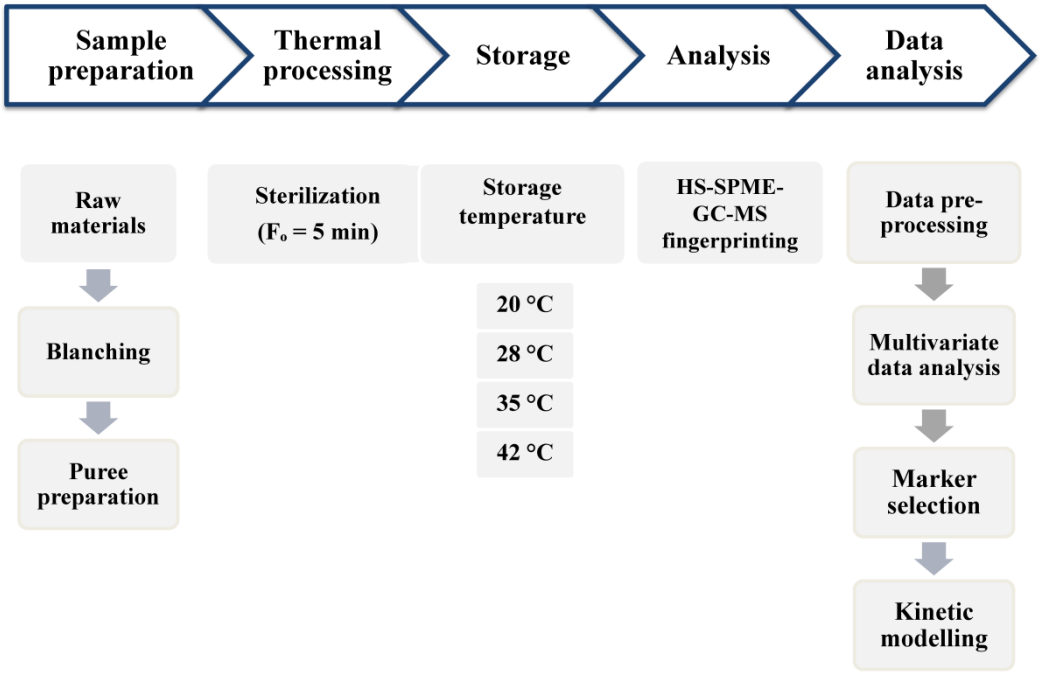


Fig. 1. Experimental set-up for investigating chemical reactions in the headspace fraction of thermally treated carrot puree using a fingerprinting-kinetics strategy.

2.2. *Thermal processing*

The thermal treatment was carried out in a static steriflow pilot retort (Barriquand, Paris, France). An industrially-relevant sterilization value of $F_{121.1^0C}^{10^0C}(F_0)$ = 5 min was selected. Glass jars (100 ml volume, 95 mm height and 45 mm diameter) were filled with 85 ± 0.5 g of carrot puree and closed with metal lids. Temperature profiles in the retort and at the coldest point of the product were recorded using type T thermocouples (Ellab, Hillerød, Denmark). The data logging device provided real-time information of the whole process. For the graphical representation of exemplary time-temperature profiles of the product and environment during treatment, the reader is referred to Kebede et al. (2013). Following completion of the treatments, samples were transferred to ice water to further cool the product down.

2.3. *Storage*

Sterilized glass jars were stored in incubators, protected from light, at 20 °C and 28 °C up to 44 weeks, at 35 °C up to 26 weeks and at 42 °C up to 18 weeks. At fixed points in time (11 points per temperature), glass jars were sampled from the incubators. The vegetable puree was aseptically (next to a Bunsen burner) transferred to small-volume (10 ml) polyethylene terephthalate tubes with a polyethylene cap. One gram of sample was taken for microbial analysis. Thereafter, the tubes were frozen in liquid nitrogen, wrapped with aluminium foil and stored in freezer at -40 °C until GC-MS analysis.

2.4. Microbial analysis

Microbial analysis was performed to verify growth of mesophilic (aerobic) and thermophilic (aerobic and anaerobic) microorganisms. Plate count agar was prepared for aerobic (mesophilic and thermophilic) bacteria, whereas the presence of anaerobic thermophiles were analyzed using reinforced clostridial agar. In the investigated shelf-life time and temperature conditions, the microbial growth was below detection limit.

2.5. *HS-SPME-GC-MS analysis*

Samples were thawed overnight in the cooling room (4 °C). 2.5 g thawed sample and 2.5 ml saturated NaCl solution were mixed into a 10 ml amber glass vial (10 ml, VWR International, Radnor, PA, USA). The vials were tightly closed using screw-caps with silicon septum seal (GRACE, Columbia, MD, USA), mixed and transferred to the cooling tray of the auto-sampler which was maintained at 10 °C. Headspace fingerprinting was conducted on a gas chromatography (GC) system (7890N, Keysight Technologies, Diegem, Belgium) coupled to a mass selective detector (MSD) (5977N, Keysight Technologies, Diegem, Belgium) and equipped with a combipal autosampler (CTC analytics, Zwingen, Switzerland). Targeting detection of a

wide range of volatiles in a particular food extract, a HS-SPME-GC-MS method of analysis was optimized beforehand (Kebede et al., 2014b). In the selected method, the samples were incubated at 40 °C during 20 minutes under agitation at 500 rpm. Next, extraction of the volatiles was performed using a HS-SPME fiber coated with 30/50 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (StableFlex, Supelco, Bellefonte, PA, USA) at 40 °C during 10 min. The SPME fiber was inserted into the heated (230 °C) GC-injection port for 2 min to desorb the volatile compounds. Prior to extraction, the fibers were conditioned and regenerated according to the manufacturer's guidelines in the conditioning station of the auto-sampler. Injection of the samples to the GC-column was performed in split (1/5) mode. Chromatographic separation was carried out on a HP-5MS capillary column (30 m ×0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies J&W, Santa Clara, CA, USA) coated with 5%-phenyl-methylpolysiloxane as a stationary phase and helium as a gas phase at a constant flow of 1.2 mL/min. The GC-oven temperature was programmed from a starting temperature of 40 °C, which was retained for 2 min, to 172 °C at 4 °C/min, then ramped to 300 °C at 30°C/min and kept constant at 300 °C for 2 min before cooling back to 40 °C. The mass spectra were obtained by electron ionization (EI) mode at 70 eV with a scanning range of 35 to 400 m/z and a scanning speed of 3.8 scans per second. MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. In this work, to minimize the phenomenon of fiber degradation, a new fiber was used for each storage temperature. Per storage temperature, during analysis, the samples were randomized as a function of storage time. Possible fiber degradation was carefully monitored by analysis of a reference sample (blanched carrot samples), every 10 injections. Per storage time and temperature condition, the analysis was repeated six times.

2.6. Data analysis

2.6.1. Data pre-processing

As commonly observed in GC-MS analysis, co-eluting compounds were present in the obtained chromatograms. Therefore, all chromatograms were analyzed with automated mass spectral deconvolution and identification system (AMDIS) (Version 2.66, 2008, National Institute of Standards and Technology, Gaithersburg, MD, USA) to extract "pure" component spectra from complex chromatograms. In addition, for proof of identity along with the mass spectral data, AMDIS was used to build a retention index calibration file. The deconvoluted spectra were then analyzed with mass profiler professional (MPP) (Version 12.0, 2012, Keysight Technologies, Diegem, Belgium) for filtering and peak alignment. The MPP yielded a spreadsheet containing peak areas, which was used as an input for the multivariate statistical data analysis.

2.6.2. Multivariate data analysis and marker selection

The multivariate data were analyzed with a multivariate data analysis (MVDA) which was carried out in Solo (Version 6.5, 2011, Eigenvector Research, Wenatchee, WA, USA). All data were mean-centered and the variables were weighed by their standard deviation to give them equal variance. In a first step, principal component analysis (PCA) was conducted as an exploratory technique to evaluate each data set and to detect potential outliers. To study the evolution during storage, per storage temperature, partial least squares (PLS) regression was performed, with the volatiles as *X*-variables and the storage time as *Y*-variable. For determining the complexity of the PLS model, the lowest number of latent variables (LVs) that maximally describe the change during storage was used. In PLS, to investigate the change in the volatile fraction as a function of time, bi-plots were plotted. Next, to select volatile compounds clearly changing during storage, variable identification (VID) coefficients were calculated. These values correspond to the correlation coefficient between each original *X*-variable and predicted (by the

selected PLS-model) *Y*-variable. In this work, variables with an absolute VID value higher than 0.70 were considered to be important. These variables were identified and linked to possible reaction pathways. Identification of the compounds was performed by comparing the deconvoluted mass spectrum with the reference mass spectra from both NIST spectral library (NIST08, version 2.0, National Institute of Standards and Technology, Gaithersburg, MD, USA) and WILEY mass spectral data (Wiley2010, version 9, Hoboken, New York, USA). For identification, a threshold match of 90 % was taken into account. For further confirmation, visual inspection of spectral matching between the detected compound and the match from the library as well as comparison of the retention index were performed.

2.6.3. Kinetic modelling and parameter estimation

Kinetic modelling was performed based on the rate equation of a degradation reaction. For a detailed discussion on the general principles of kinetic modeling, the reader is referred to the work of van Boekel (2009).The general rate equation of an n^{th} order degradation reaction is expressed as **Equation 1**, where v is the reaction rate, A is any property of interest, n is the reaction order and k is the rate constant. Volatiles that were changing during storage could be modelled best by a first-order kinetic model (n = 1)**,** where A_0 is the initial concentration at time t = 0 (start of storage) and t is the storage time in weeks (**Equation 2**). The temperature dependency of the reaction rate constant was evaluated using the Arrhenius equation (**Equation 3**), where E_a is the activation energy (kJ/mol), T is the storage temperature in kelvin, k_{ref} is the rate constant (weeks^{-1}) at reference storage temperature (20 °C) and R is the gas constant (kJ/mol*k).

$$v = \frac{\mathrm{dA}}{\mathrm{d}t} = -kA^n \quad (1)$$

$$A = A_0\, exp(-kt) \quad (2)$$

183 $$k = k_{ref} exp\left(\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad (3)$$

184 Model evaluation was performed by examining $R^2_{adjusted}$ (**Equation 4**) and by visual inspection of
185 the parity plot (estimated values versus measured values) and the scatter plot (residuals versus
186 estimated values). In **Equation 4**, Where DF_{tot} and DF_{error} are degree of freedom of total and
187 error, respectively and SS is the sum of squares:

188 $$R^2_{adjusted} = 1 - \frac{\left[(DF_{tot}-1)\left(1-\frac{SS_{model}}{SS_{total}}\right)\right]}{DF_{error}} \quad (4)$$

189

190 One-step regression analysis was performed by inserting **Equation 3 in Equation 2**, using SAS®
191 software (version 9.3, Cary, USA).

192 ## 3. RESULTS AND DISCUSSION

193 As discussed in the introduction, the present work focuses on investigating chemical reactions
194 during shelf-life using an integrated analytical and engineering toolbox: called "fingerprinting-
195 kinetics". In the first step, the potential of fingerprinting was used as fast screening technique for
196 monitoring chemical changes in thermally treated carrot puree as a function of storage.
197 Fingerprinting enabled selection of volatiles of which detected amounts are changing the most
198 during shelf-life. Only these volatiles were identified and further zoomed into (to link them to
199 possible reaction pathways) (section 3.1). In the next step, kinetics was performed to increase
200 insight into the storage temperature dependency of reaction kinetics of volatiles changing as a
201 function of shelf-life. By evaluating the estimated kinetic parameters, the suitability of these
202 volatiles as quality indices (markers) for ASLT was investigated (section 3.2).

3.1. Headspace SPME-GC-MS fingerprinting

As discussed in section 2.4, in order to detect a wide range of volatiles, a HS-SPME–GC–MS fingerprinting procedure was optimized. As more than 100 headspace components were detected per storage temperature and compounds are unknowns at the first instance, the procedure can be considered as untargeted for the analyzed particular extract. **Fig. 2** depicts an exemplary GC-MS total ion chromatogram of the headspace of thermally treated carrot puree at the start of storage (day 0). As described in section 2.5.1, the complex GC–MS data files were analyzed with a sequence of data preprocessing techniques (i.e., AMDIS and MPP). The MPP obtained a spreadsheet containing peak areas, which was used as an input for the next statistical analysis (MVDA).

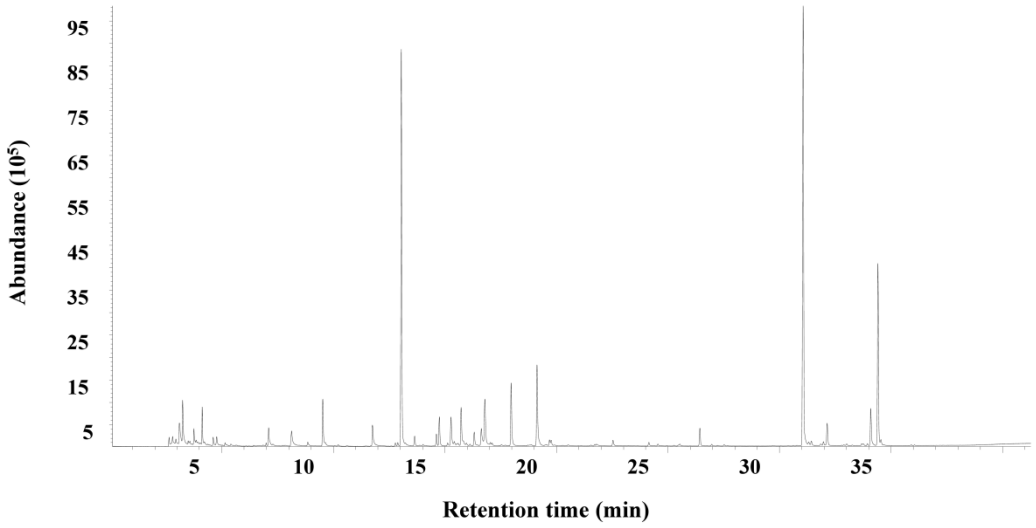
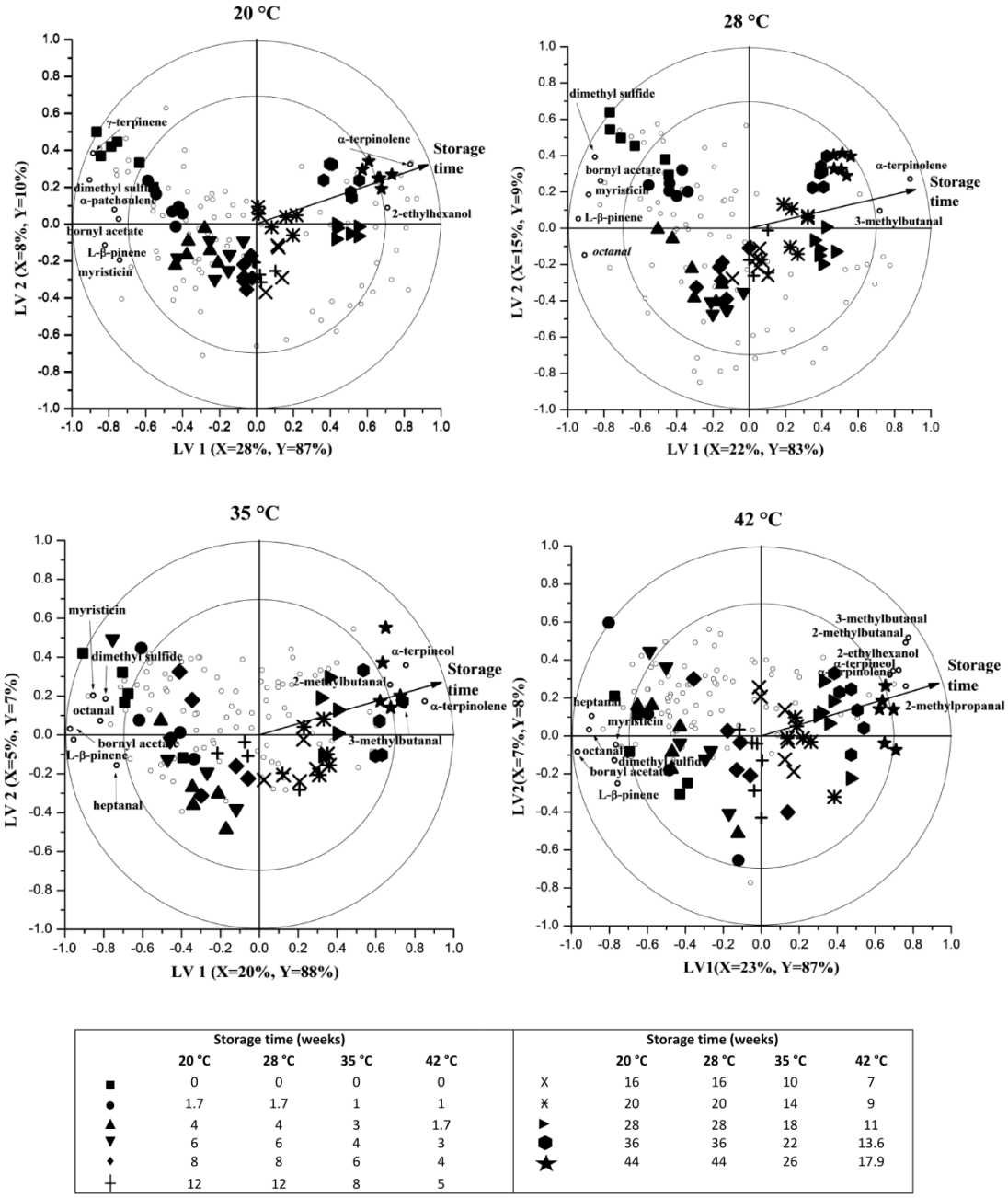


Fig. 2. Total ion chromatogram of the headspace of thermally treated carrot puree at the start of storage (day 0), obtained by headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting.

After exploring the data with PCA (data not shown), PLS regression was conducted per storage temperature with the carrot headspace components considered as *X*-variables and storage time as continuous *Y*-variable. For each storage temperature, the first two latent variables (LVs)

explained a considerable amount of the *Y* variance (97 %, 93 %, 95 % and 95 % for 20 °C, 28 °C, 35 °C and 42 °C, respectively), with a large portion already covered by the first LV (**Fig. 3)**.



Storage time (weeks)				
	20 °C	28 °C	35 °C	42 °C
■	0	0	0	0
●	1.7	1.7	1	1
▲	4	4	3	1.7
▼	6	6	4	3
♦	8	8	6	4
+	12	12	8	5

Storage time (weeks)				
	20 °C	28 °C	35 °C	42 °C
X	16	16	10	7
✱	20	20	14	9
►	28	28	18	11
⬢	36	36	22	13.6
★	44	44	26	17.9

Fig. 3. PLS-bi-plots describing the effect of storage on the volatile fraction of thermally treated carrot puree (objects represented by differently shaped symbols) stored at different temperatures, i.e., 20 °C, 28 °C, 35 °C, and 42 °C. The open circles represent headspace components, of which only components selected by the VID procedure are identified and marked in bold (Table 2). The vectors represent the correlation loading for the *Y*-variable (time). The percentages of the *X*- and *Y*-variances explained by each latent variable (LV1 and LV2) are indicated on the respective axes.

Accordingly, for each storage temperature, a multivariate PLS model based on two LVs was selected. To visualize the multidimensional data structure, bi-plots were constructed per storage temperature (**Fig. 3**). A bi-plot is an interesting tool to graphically represent the change in the multidimensional data set, i.e. in the headspace fractions during storage.

As can be seen from **Figure**, the trend of storage time can be clearly observed on the bi-plots. The first clear trend is the horizontal projection of the volatile fractions of the carrot purees from the left of the bi-plot to the right of the bi-plot. This dominant change during storage is described by the first LV, as indicated in the respective axis. The second trend is the u-shaped structure of the Y-variables (volatile fractions). This shows that there is also a variation in the vertical direction in addition to the horizontal direction. This second variation is described by the second LV. The bi-plots also display the relation between headspace compounds and storage time. For instance, compounds that are located in the same direction as the *Y*-vector (the vectors represent the correlation loading for the *Y*-variable (time)) are positively correlated with increasing storage time indicating increase in concentration as a function of storage time, while the ones that are projected in the opposite direction are negatively correlated and decrease with storage time. For samples stored at storage temperatures of 20 °C, 28 °C and 35 °C, most of the components (small open circles, **Fig. 3**) are projected to the beginning of shelf-life while fewer are positioned close to the end of shelf-life. This seems to suggest that the concentration of most of these compounds have decreased during storage while only few compounds appear to be formed. Nevertheless, more compounds seem to be formed at 42 °C compared to the other storage temperatures. This phenomenon is further explained in section 3.2. In addition, on the bi-plots, based on the distance of a component from the center of the coordinate, its importance for displaying the changes during storage can be discussed. For instance, if a compound is projected far from the center and located between the two ellipses (inner and outer ellipses represent

correlation coefficients of 70 and 100 %, respectively) on the plot, this shows that the concentration of this compound has largely changed as function of storage time.

Table 1

Volatiles significantly changing as a function of shelf-life, per storage temperature (20 °C, 28 °C, 35 °C and 42 °C), selected based on the VID procedure, listed in increasing order of VID coefficient. Positive VID coefficients signify an increase in concentration during storage while negative coefficients denote a decrease. The retention index (RI) and possible chemical group is also listed.

Storage temperature	VID	Identity	RI	Chemical group
20 °C	-0.85	dimethyl sulfide	723	Sulfur compound
	-0.83	L-β-pinene	1055	Terpene
	-0.81	γ-terpinene	1148	Terpene
	-0.76	myristicin	1696	Phenylpropanoids
	-0.74	α-patchoulene	1701	Terpene
	-0.73	bornyl acetate	1391	Terpene
	0.71	2-ethylhexanol	1113	Alcohol
	0.88	α-terpinolene	1074	Terpene
28 °C	-0.91	octanal	1083	Aldehyde (aliphatic)
	-0.90	L-β-pinene	1055	Terpene
	-0.81	myristicin	1696	Phenylpropanoids
	-0.72	bornyl acetate	1391	Terpene
	-0.71	dimethyl sulfide	723	Sulfur compound
	0.72	3-methylbutanal	763	Aldehyde (Strecker)
	0.93	α-terpinolene	1074	Terpene
35 °C	-0.96	bornyl acetate	1391	Terpene
	-0.95	L-β-pinene	1055	Terpene
	-0.81	myristicin	1696	Phenylpropanoids
	-0.80	octanal	1083	Aldehyde (aliphatic)
	-0.76	dimethyl sulfide	723	Sulfur compound
	-0.75	heptanal	970	Aldehyde (aliphatic)
	0.70	2-methylbutanal	768	Aldehyde (Strecker)
	0.76	3-methylbutanal	763	Aldehyde (Strecker)
	0.80	α-terpineol	1293	Terpene-alcohol
	0.87	α-terpinolene	1075	Terpene
42 °C	-0.97	L-β-pinene	1056	Terpene
	-0.89	octanal	1084	Aldehyde (aliphatic)
	-0.86	heptanal	970	Aldehyde (aliphatic)
	-0.79	dimethyl sulfide	723	Sulfur compound
	-0.78	bornyl acetate	1391	Terpene
	-0.76	myristicin	1696	Phenylpropanoids
	0.72	α-terpinolene	1075	Terpene
	0.74	α-terpineol	1293	Terpene-alcohol
	0.77	2-ethylhexanol	1113	Alcohol
	0.79	2-methylpropanal	731	Aldehyde (Strecker)
	0.83	2-methylbutanal	768	Aldehyde (Strecker)
	0.85	3-methylbutanal	763	Aldehyde (Strecker)

Even though the bi-plot provides graphical representation of the changes, it is not straightforward to rank the relative component importance for the change as a function of storage time. Therefore, to quantitatively rank volatile's importance for the change, VID coefficients were calculated using the selected PLS models (Section 2.6.2). Each volatile was assigned with a value between -1 and +1, where a positive VID coefficient represents a higher concentration after storage and *vice versa*. Since the objective was to determine compounds highly changing in concentration, only those with absolute value higher than 0.70 were selected to further zoom into and only those were identified (**Table 1; Fig. 3** (bold open circles)). In samples stored at storage temperatures of 20 °C, 28 °C, 35 °C and 42 °C, respectively, 8, 7, 10 and 12 volatile compounds were selected.

To increase insight into the effect of storage time on quality-related chemical reactions, it was tried to link the significantly changing volatiles to possible reaction pathways. In this work, the selected compounds (**Table 1**) can be categorized under terpenoids (L-β-pinene, γ-terpinene, α-patchoulene, α-terpinolene, bornyl acetate and α-terpineol), phenylpropanoids (myristicin), aldehydes and alcohols (octanal, heptanal, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal and 2-ethylhexanol) and sulfur-containing (dimethyl sulfide) chemical classes. Prior to thermal sterilization, the carrot cubes were blanched (section 2.1). In addition, from the microbial analysis (section 2.4), no spoilage was detected in the investigated shelf-life time-scale and storage temperatures. Therefore, enzymatic and microbial activities were not expected to have a significant impact on the change in volatiles. In that context, in carrots, the changes of the selected volatiles can be linked to non-enzymatic and non-microbial chemical reactions such as terpenoid and phenylpropanoid degradation, unsaturated fatty acid degradation, Strecker degradation and degradation of sulfur-containing amino acids, respectively. In the following paragraphs, these effects will be discussed step by step in more detail.

On one hand, the concentrations of the majority of terpenoids have decreased during storage, possibly due to degradation into other components. It has been previously described that these compounds are highly thermolabile and sensitive even to low temperature conditions (Heatherbell, Wrolstad, & Libbey, 1971; Shamaila, Durance, & Girard, 1996). On the other hand, the amount of two other monoterpenes (e.g. α-terpinolene and α-terpineol) increased during storage. In literature, formation of α-terpineol, which is terpene alcohol, is linked to oxidative processes, in which the tetra-substituted terpenes double bond is attacked (Kjeldsen, Christensen, & Edelenbos, 2003; Jones, 2008). It can be hypothesized that during storage oxidative conversion of some terpenes to terpene alcohols occurs. With respect to α-terpinolene, the literature search didn't reveal a possible explanation for its increased formation during storage at all storage temperatures. Interpreting the selected volatiles and reactions in the context of sensory aspects, the reduction in genuine terpenes might magnify the desired sweet carrot flavor through reducing the harsh or burning-like flavor, which is mostly associated with elevated terpene concentration. Nevertheless, in case of their excessive reduction, the characteristic carrot aroma may be significantly affected (Howard, Braswell, Heymann, Lee, Pike, & Aselage, 1995; Kjeldsen et al., 2003).

The other group of selected chemical compounds are aliphatic aldehydes: octanal and heptanal, which are mostly linked with autoxidation and/or thermally induced oxidation of unsaturated fatty acids (Heatherbell et al., 1971; Kebede et al., 2013; Kebede et al., 2014a; Kebede et al., 2014b). The first stage of these oxidative reactions involves uptake of oxygen in the presence of catalysts, such as transition metals, which is initiated by heat or light. Once highly reactive free radicals are formed, they react in auto-catalytic mode to generate a complex mixture of low molecular weight compounds. Heptanal and octanal have been detected before as volatiles in

cooked carrots and were reported to increase considerably at high process temperatures (Buttery, Seifert, Guadagni, Black, & Ling, 1968; Vervoort et al., 2013; Buttery & Takeoka, 2013). In this work, even though these volatiles are increasingly detected after thermal sterilization, their concentration decreased during storage. It can be hypothesized that after processing, formation of these compounds halted and during storage oxidative breakdown of the aldehydes into other compounds such as aliphatic alcohols (e.g., 2-ethylhexanol) occurred, using the residual oxygen in the system. From sensorial point of view, a certain level of these aliphatic aldehydes and alcohols is generally considered necessary to give characteristic odor and flavor properties to foods, but since many of these aldehydes and alcohols give rise to typical rancid state of flavors, an ideal balance has to be achieved in foods.

As can be seen from **Table 1**, Strecker aldehydes, such as 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, were selected with positive VID values. These volatiles are reaction products of Strecker degradation, one of the side reactions of the Maillard reaction. Although Strecker degradation is a sub-reaction category of the Maillard reaction scheme, it has been described to direct the Maillard reaction towards the aromagenic pathways rather than to chromogenic pathways. In other words, this reaction is of outermost importance in relation to flavor formation (Yaylayan, 2003; van Boekel, 2006; Rizzi, 2008). In our previous studies, comparing the impact of thermal and high pressure high temperature sterilization immediately after processing, Strecker aldehydes were detected at higher levels in a wide range of thermally sterilized vegetable purees (Kebede et al., 2013; Kebede et al., 2014a; Kebede et al., 2014b). However, little information is available regarding the evolution during storage. The present work demonstrates that these compounds further increased as a function of storage. Therefore, it can be hypothesized that the Maillard reaction and its side reactions seem to take place also during storage of thermally sterilized carrot puree.

Dimethyl sulfide is known to be formed from S-methylcysteine, which is a highly thermolabile sulfur containing amino acid derivatives. Previous studies (Vervoort et al., 2013; Kebede et al., 2013; Kebede et al., 2014a; Kebede et al., 2014b) reported an increased decomposition of S-methylmethionine after thermal sterilization leading to the formation of these compounds which are responsible for a typical canned flavor (Bills & Keenan, 1968; Heatherbell et al., 1971; Kubec, Drhova, & Velisek, 1998; Araya et al., 2009). In line with the above discussions, in the present work, dimethyl sulfide was detected at higher amount after thermal sterilization. However, the concentrations decreased during storage, which may be attributed to oxidative degradation reactions during storage.

In general it was shown that, per storage temperature, fingerprinting enabled selection of volatile compounds and reactions significantly changing as a function of shelf-life. As discussed before as a second objective in this work, the suitability of these selected volatiles as quality indices (markers) for ASLT was investigated. Based on the data from the fingerprinting as a function of times and temperatures, kinetic modelling of the selected volatiles was performed to investigate their reaction kinetics at different storage temperatures (section 3.2).

3.2. Reaction kinetics of the changes of headspace components

Firstly, an appropriate kinetic model was identified. Next, kinetic parameters, such as reaction rate constants and activation energies, were estimated using a non-linear one-step regression analysis (inserting **Equation 3 in Equation 2**). Of all selected compounds, γ-terpinene, α-patchoulene and 2-ethylhexanol showed a scattering behaviour as a function of storage time, specifically at higher storage temperatures (results not shown). This might be an indication for the presence of non-elementary complex chemical reactions behind the formation of these

products. In addition, the reactions seem to be temperature independent. Hence, these compounds don't seem interesting for ASLT and are not modelled at this stage.

Table 2

Estimated kinetic parameters based on a one-step first-order kinetic model, Equation 2 & 3, (20 °C as reference temperature) describing changes during storage of discriminant headspace components in thermally treated carrot puree. Samples were stored at 20 °C, 28 °C, 35 °C and 42 °C

Compound	k_{ref} (week^{-1})	Ea (kJ/mol)	r^2_{adj}
dimethyl sulfide	0.112 ± 0.014	14 ± 6	0.96
bornyl acetate	0.050 ± 0.009	15 ± 9	0.93
myristicin	0.044 ± 0.006	37 ± 7	0.96
α-terpinolene	-0.020 ± 0.004	49 ± 7	0.87
L-β-pinene	0.015 ± 0.002	72 ± 5	0.99
α-terpineol	-0.009 ± 0.005	94 ± 17	0.76
octanal	0.015 ± 0.004	96 ± 11	0.94
2-methylbutanal	-0.004 ± 0.001	96 ± 10	0.97
heptanal	0.005 ± 0.002	108 ± 16	0.95
2-methylpropanal	-0.003 ± 0.002	113 ± 15	0.94
3-methylbutanal	-0.002 ± 0.001	121 ± 17	0.98

The change in the concentration of the rest of the terpenes could be modelled best by means of a first-order empirical kinetic model. The formation of the Strecker aldehydes could be modelled best by means of a zero-order empirical kinetic model. The kinetic parameters and their corresponding 95 % approximate confidence interval are listed in **Table 2**. As an example, the changes of L-β-pinene, 2-methylbutanal and octanal are presented in **Fig. 4**. In the top section, compound's peak area as a function of time in thermally treated carrot puree stored at 20 °C, 28 °C, 35 °C and 42 °C is shown. The full lines represent peak area predicted by the kinetic model while the experimental data are represented by the symbols. The model was evaluated, as described in section 2.5.3, using parity plot, scatter plot and $R^2_{adjusted}$. In the present work, the observed kinetic parameters are empirical, and thus not necessarily reflecting the actual reaction mechanism. They are, however, a useful tool to obtain insight into the impact of storage on compound changes.

For determining which of these volatiles (from **Table 2**) could be potential ASLT markers, two criteria were established: (i) the reaction should be temperature-dependent and (ii) there should be an observable change not only at temperature-abuse conditions but also at reference/ambient storage temperature (20 °C). Dimethyl sulfide and bornyl acetate are characterized by very low E_a-values compared to other compounds, indicating a very low temperature dependency. Hence, given the very small reaction acceleration by increasing storage temperature, these volatiles seem less interesting to be considered as markers for ASLT. For the rest of the compounds, as can be seen from their E_a-values, increasing the storage temperature effectively increased the rate constants. Nevertheless, for some of them (e.g., heptanal, 2-methylbutanal, 2-methylpropanal and 3-methylbutanal) the formation at 20 °C proceeds very slow and seem to largely increase at an elevated storage temperature, as indicated by their relatively high activation energies (**Table 2**). As also discussed in section 3.1, these compounds were selected by the VID procedure at all storage temperatures but not at 20 °C, indicating the strong temperature dependency of the reaction kinetics. Therefore, taking into account the second criteria established in this work, care should be taken if and when considering these temperature sensitive volatiles as ASLT markers.

Considering both established criteria, myristicin, α-terpinolene, L-β-pinene, α-terpineol and octanal seem to be interesting compounds. Their reaction follows Arrhenius kinetics wherein higher storage temperatures lead to the acceleration of the rate of the reaction. In addition, their rate constant is also significant at ambient storage temperature. Therefore, these volatiles compounds can be considered as potential markers for ASLT of thermally sterilized carrot puree.

4. CONCLUSION

This study clearly showed the power of the integrated fingerprinting-kinetics approach to increase insight into chemical changes during shelf-life of thermally sterilized carrot purees and

to select markers for ASLT. In a first step, fingerprinting enabled selection of headspace compounds significantly changing during shelf-life. The selected compounds can be categorized into Strecker aldehydes, terpenoids, fatty acid derivatives and sulfur-containing compounds. The concentrations of Strecker aldehydes increased during storage, whereas the majority of terpenes seem to decrease during storage. In the next step, the suitability of these volatiles as markers for accelerated shelf-life testing (ASLT) was investigated. By evaluating the estimated kinetic parameters, myristicin, α-terpinolene, L-β-pinene, α-terpineol and octanal were selected as potential markers for ASLT of thermally sterilized carrot puree. In literature, it has been reported that the characteristic aroma and flavor of a carrot is mainly influenced due to terpenes. The fact that the research strategy followed in the present work enabled the selection of terpenes (except octanal) as markers give an indication that ASLT can be used for shelf-life evaluation and estimation of sterilized carrot samples.

By evaluating the estimated kinetic parameters, myristicin, α-terpinolene, L-β-pinene, α-terpineol and octanal were selected as potential markers for ASLT of thermally sterilized carrot puree. In general, the applicability of a marker (selected by the fingerprinting-kinetics strategy) to be used for shelf-life estimation can be categorized into three. (i) In cases the marker determines the best before date of the food product and is important for the consumer, the kinetics can be directly used to shelf-life estimation. (ii) If the marker does not directly determine the best before date, but is linked to a reaction causing shelf-life changes, it has a potential to be used as a witness for shelf-life changes and also for shelf-life estimation. (iii) If the latter is not the case, but the kinetics of the marker matches with kinetics of a compound determining the best before date, it has still a potential to be used for ASLT. Based on the obtained results, it is difficult to evaluate to which extent the selected markers affect overall carrot flavor. This was not the aim of the present work, but in the future it is worthwhile to perform a sensory analysis, to understand how the observed changes will be appreciated. In

addition, the impact on different extract of the volatile fraction or on other fraction (e.g. liquid fraction) should be investigated to link the observed changes to other quality attributes, such as vitamins and color.

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6. References

Adebooye, O. C., Vijayalakshmi, R., & Singh, V. (2008). Peroxidase activity, chlorophylls and antioxidant profile of two leaf vegetables (Solanum nigrum L. and Amaranthus cruentus L.) under six pretreatment methods before cooking. *International Journal of Food Science and Technology, 43*(1), 173-178.

Araya, X. I. T., Smale, N., Zabaras, D., Winley, E., Forde, C., Stewart, C. M., & Mawson, A. J. (2009). Sensory perception and quality attributes of high pressure processed carrots in comparision to raw, sous-vide and cooked carrots. *Innovative Food Science & Emerging Technologies, 10*(1), 420-433.

Bills, D. D. & Keenan, T. W. (1968). Dimethyl sulfide and its precursor in sweet corn. *Journal of Agricultural and Food Chemistry, 16*(4), 643-645.

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Black, D. R., & Ling, L. (1968). Characterization of some volatile constituents of carrots. *Journal of Agricultural and Food Chemistry, 16*(6), 1009-1015.

Buttery, R. G. & Takeoka, G. R. (2013). Cooked Carrot Volatiles. AEDA and Odor Activity Comparisons. Identification of Linden Ether as an Important Aroma Component. *Journal of Agricultural and Food Chemistry, 61*(38), 9063-9066.

Corradini, M. G. & Peleg, M. (2007). Shelf-life estimation from accelerated storage data. *Trends in Food Science & Technology, 18*(1), 37-47.

Grauwet, T., Vervoort, L., Colle, I., Van Loey, A., & Hendrickx, M. (2014). From fingerprinting to kinetics in evaluating food quality changes. *Trends in Biotechnology, 32*(3), 125-131.

Heatherbell, D. A., Wrolstad, R. E., & Libbey, L. M. (1971). Carrot Volatiles .1. Characterization and Effects of Canning and Freeze Drying. *Journal of Food Science, 36*(2), 219-224.

Hough, G., Garitta, L., & Gomez, G. (2006). Sensory shelf-life predictions by survival analysis accelerated storage models. *Food Quality and Preference, 17*(6), 468-473.

Howard, L. R., Braswell, D., Heymann, H., Lee, Y., Pike, L. M., & Aselage, J. (1995). Sensory Attributes and Instrumental Analysis Relationships for Strained Processed Carrot Flavor. *Journal of Food Science, 60*(1), 145-148.

Jones, M. J. (2008). Formation of vegetable flavour. In B. Brückner & S. Grant Wyllie, *Fruit and vegetable flavour Recent advances and future prospects* (pp. 71-96). Cambridge: Woodhead Publishing Limited.

Kebede, B. T., Grauwet, T., Mutsokoti, L., Palmers, S., Vervoort, L., Hendrickx, M., & Van Loey, A. (2014a). Comparing the impact of high pressure high temperature and thermal sterilization on the volatile fingerprint of onion, potato, pumpkin and red beet. *Food Research International, 56*(0), 218-225.

478 Kebede, B. T., Grauwet, T., Palmers, S., Vervoort, L., Carle, R., Hendrickx, M., & Van Loey, A.
479 (2014b). Effect of high pressure high temperature processing on the volatile fraction of
480 differently coloured carrots. *Food Chemistry, 153*(0), 340-352.

481 Kebede, B. T., Grauwet, T., Tabilo-Munizaga, G., Palmers, S., Vervoort, L., Hendrickx, M., &
482 Van Loey, A. (2013). Headspace components that discriminate between thermal and high
483 pressure high temperature treated green vegetables: Identification and linkage to possible
484 process-induced chemical changes. *Food Chemistry, 141*(3), 1603-1613.

485 Kjeldsen, F., Christensen, L. P., & Edelenbos, M. (2003). Changes in volatile compounds of
486 carrots (Daucus carota L.) during refrigerated and frozen storage. *Journal of Agricultural and*
487 *Food Chemistry, 51*(18), 5400-5407.

488 Kubec, R., Drhova, V., & Velisek, J. (1998). Thermal degradation of S-methylcysteine and its
489 sulfoxide - Important flavor precursors of Brassica and Allium vegetables. *Journal of*
490 *Agricultural and Food Chemistry, 46*(10), 4334-4340.

491 Labuza, T. P. & Taoukis, P. S. (1990). The relationship between processing and shelf-life. In G.
492 G. Birch, G. Campbell-Plat, & M. G. Lindley, *Foods for the 90's* (pp. 73-105). New York:
493 Elsevier Applied Science.

494 Mizrahi, S. (2000). Accelerated shelf-life tests. In D. Kilcast & P. Subramaniam, *The stability*
495 *and shelf-life of food* (pp. 107-125). Cambridge: Woodhead Publishing Limited and CRC press
496 LLC.

497 Rizzi, G. P. (2008). The strecker degradation of amino acids: Newer avenues for flavor
498 formation. *Food Reviews International, 24*(4), 416-435.

499 Robertson, G. L. (2000). Shelf-Life of Packaged Foods, Its Measurment and Prediction. In G. L.
500 Robertson, *Food Packages: Principles and Practices*). New York: CRC press Inc.

501 Saavedra, J., Cordova, A., Galvez, L., Quezada, C., & Navarro, R. (2013). Principal Component
502 Analysis as an exploration tool for kinetic modeling of food quality: A case study of a dried
503 apple cluster snack. *Journal of Food Engineering, 119*(2), 229-235.

504 Shamaila, M. A. W. E., Durance, T. I. M. O., & Girard, B. E. N. O. (1996). Water Blanching
505 Effects on Headspace Volatiles and Sensory Attributes of Carrots. *Journal of Food Science, 61*,
506 1191-1195.

507 Sithole, R., McDaniel, M. R., & Goddik, L. M. (2005). Rate of Maillard Browning in Sweet
508 Whey Powder. *Journal of Dairy Science, 88*(5), 1636-1645.

509 van Boekel, M., Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., Lalljie, S., Somoza, V.,
510 Knorr, D., Jasti, P. R., & Eisenbrand, G. (2010). A review on the beneficial aspects of food
511 processing. *Molecular Nutrition & Food Research, 54*(9), 1215-1247.

512 van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction.
513 *Biotechnology Advances, 24*(2), 230-233.

van Boekel, M. A. J. S. (2009). *Kinetic Modeling of Reactions in Foods*. CRC PRESS-TAYLOR & FRANCIS GROUP, 6000 BROKEN SOUND PARKWAY NW, STE 300, BOCA RATON, FL 33487-2742 USA.

Vervoort, L., Grauwet, T., Kebede, B. T., Van der Plancken, L., Timmermans, R., Hendrickx, M., & Van Loey, A. (2012). Headspace fingerprinting as an untargeted approach to compare novel and traditional processing technologies: A case-study on orange juice pasteurisation. *Food Chemistry, 134*(4), 2303-2312.

Vervoort, L., Grauwet, T., Njoroge, D. M., Van der Plancken, I., Matser, A., Hendrickx, M., & Van Loey, A. (2013). Comparing thermal and high pressure processing of carrots at different processing intensities by headspace fingerprinting. *Innovative Food Science & Emerging Technologies, 18*, 31-42.

Yaylayan, V. A. (2003). Recent Advances in the Chemistry of Strecker Degradation and Amadori Rearrangement: Implications to Aroma and Color Formation. *Food Science and Technology Research, 9*(1), 1-6.